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Coupling of metal-organic frameworks-containing monolithic capillary-based selective enrichment with matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for efficient analysis of protein phosphorylation☆

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ABSTRACT

Protein phosphorylation is a major post-translational modification, which plays a vital role in cellular signaling of numerous biological processes. Mass spectrometry (MS) has been an essential tool for the analysis of protein phosphorylation, for which it is a key step to selectively enrich phosphopeptides from complex biological samples. In this study, metal-organic frameworks (MOFs)-based monolithic capillary has been successfully prepared as an effective sorbent for the selective enrichment of phosphopeptides and has been off-line coupled with matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) for efficient analysis of phosphopeptides. Using š-casein as a representative phosphoprotein, efficient phosphorylation analysis by this off-line platform was verified. Phosphorylation analysis of a nonfat milk sample was also demonstrated. Through introducing large surface areas and highly ordered pores of MOFs into monolithic column, the MOFs-based monolithic capillary exhibited several significant advantages, such as excellent selectivity toward phosphopeptides, superb tolerance to interference and simple operation procedure. Because of these highly desirable properties, the MOFs-based monolithic capillary could be a useful tool for protein phosphorylation analysis.

1. Introduction

Protein phosphorylation is one of the most common and most important post-translational modifications (PTMs) in mammalian species, which plays vital important role in many biological processes, such as cell growth, migration, division, and intercellular communication [1,2]. Mass spectrometry (MS) has been an indispensable technique for phosphorylation analysis in proteome-wide due to its high sensitivity, high-throughput, feasibility for identification of phosphorylation sites and quantification of changes in phosphorylation states. However, it often fails to identify phosphopeptides in complex peptide mixtures generated by phosphoprotein digestion, because it often suffers from low abundance of phosphopeptides, poor ionization efficiency, and signal suppression by high-abundance non-phosphopeptides. Therefore, selective enrichment is usually a critical step for the analysis of phosphopeptides from complex peptide mixtures [3,4].

To date, a number of approaches, including immunoprecipitation [5–7], chemical coupling [8,9], ion exchange chromatography [10–12], metal oxide affinity chromatography (MOAC) [13–17], immobilized metal ion affinity chromatography (IMAC) [18–26], molecularly-imprinted polymers (MIPs) [27–30], and commercial non-IMAC affinity enrichment kits (e.g., MassPREP enrichment kit from Waters), have been developed for the selective enrichment of phosphoproteins/phosphopeptides. Among them, IMAC is one of the most commonly used techniques for selective isolation of phosphorylated peptides from predominantly non-phosphorylated peptides due to fast response, low cost and easy handling. The principle of IMAC is that metal ions (e.g., Fe^{3+} and Ti^{4+}) immobilized on supporting materials (e.g., silica, polymer, magnetic nanoparticle andmesoporous bead) form coordination with phosphate ions at an acidic pH (trifluoroacetic acid) while the coordination is disrupted when an elution solution of ammonia solution is applied. The selectivity of IMAC toward phosphopeptides is based on the high affinity of the phosphate groups with the metal ions bound on sorbents through iminodiacetic acid (IDA),

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nitrilotriacetic acid (NTA), polydopamine or phosphate groups [18,22–25,31–34]. However, the bound metal ions using traditional linkers such as IDA and NTA were easily lost during the sample loading and washing procedure due to the relatively weaker interaction, which greatly reduced the enrichment efficiency. Zou and co-workers had introduced new linkers to immobilize metal ions to overcome the above drawback, and had successfully applied the obtained IMAC materials for phosphoproteome analysis [22,24,35–44].

In recent years, porous IMAC materials also have been widely applied in phosphopeptides researches due to its merits of large surface area, unique pore volume, and regular porous structure [38,41,45–47]. To this end, metal-organic frameworks (MOFs), as a type of ordered porous materials, could be a new useful sorbent. Because of their super high porosity, well-defined porous structure and super large surface areas, MOFs have shown great potentials for storage [48], separation [49–52], sensing [53], drug delivery [54] and catalysis [55,56]. Recently, it has been reported that MOFs could function as sorbents for the separation and enrichment of phosphopeptides owing to the presence of plenty of Lewis acid sites [57,58]. However, MOFs in its native format are not ideal materials since their use in sample treatment usually requires centrifugation at high speed, which makes the enrichment process laborious and inconvenient. MOFs-containing magnetic materials have been a solution to this issue. Magnetic microspheres modified with zirconium-based MOFs [59] and magnetic nanoparticles with iron-based MOFs [60] have been developed for phosphopeptides enrichment. However, MOFs in other formats for sample treatment have not been well explored.

Monolithic columns [61–64], which are defined as "continuous stationary phases that form as a homogeneous column in a single piece", have been efficient media for chromatographic separation and sample treatment. Monolithic capillary can provide several significant advantages, such as ease of preparation, low cost, low back pressure, fast convective mass transfer, low sample consumption, convenient online coupling to MS and easy post-modification. Particularly, monolithic capillary has been demonstrated to be an efficient and convenient platform for coupling with MS for proteomic analysis [12,65]. Therefore, MOFs in the format of monolithic column would be efficient media for separation and sample pretreatment. Recently, several MOFs-based monolithic columns have been reported [66–68]. However, these MOFs-based monolithic columns relied on powder-packing [66] or MOFs-doping synthesis [67,68]. The powder-packing synthetic method could not provide as well-defined porous structure as classic MOFs, while MOFs-doping synthesis reduced not only the accessibility of MOFs but also the mechanical strength of resultant monolithic columns. Therefore, it is very necessary to develop MOFs-based monolithic columns that can avoid the above disadvantages for selective enrichment of phosphopeptides.

Herein, we synthesized a MOFs-based monolithic capillary through copolymerization and established a hyphenated approach of MOFs-monolithic capillary-based selective enrichment with matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) MS for highly efficient phosphorylation analysis. The synthetic procedure of the MOFs-based monolithic capillary is illustrated in Fig. 1A. It included three simple steps: (1) synthesis of amino groups functionalized MOFs (UiO-66-NH_2); (2) synthesis of butyl methacrylate (UiO-66-NH-Met), in which UiO-66-NH_2 was reacted with methacrylic anhydride; (3) preparation of MOFs-based monolithic capillary, in which UiO-66-NH-Met was copolymerized with cross-linker to form MOFs-based monolithic column. The porous structure of MOFs and the large amount of coordinated Zr (IV) endow the material with numerous functional spots for large enrichment capacity and high selectivity for phosphopeptides. In addition, the material structure format of monolithic capillary can provide nanoliter-scaled sample processing capability, online operation possibility and good reproducibility. More importantly, because of the use of copolymerization method, the MOFs-based monolithic capillary could provide highly accessible binding sites to phosphopeptides. Thus, the MOFs-based monolithic capillary could be a useful extraction tool for the selective enrichment of phosphopeptides. By coupling this monolithic capillary with MALDI-TOF MS, a novel platform was established for efficient protein phosphorylation analysis. As depicted in Fig. 1B, the procedure of the approach included four steps: sample loading, washing, eluting, and MS analysis. The MOFs-based monolithic capillary exhibited excellent selectivity, superb tolerance to interference and simple operation procedure. Highly efficient phosphorylation analysis was demonstrated by the analysis of tryptic digestion of standard protein and nonfat milk. Although previously reported *in situ* enrichment of phosphopeptides on MALDI plates could simplify the analysis of phosphopeptides due to the combination of selective enrichment and direct detection on the target plate [69–75], the presented approach can provide several advantages such as large detectability, online operation possibility, and good reproducibility over the current techniques due to the combination of MOFs with monolithic capillary.

2. Experimental

2.1. Materials and characterization

Trypsin, š-casein, bovine serum albumin (BSA), acetonitrile (ACN) and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). γ-Methacryloxypropyltrimethoxysilane (γ-MAPS), sodium hydroxide, poly (ethylene glycol) diacrylate (PEGDA, average molecular weight 256), poly (ethylene glycol) (PEG, Mn = 200), trifluoroacetic acid (TFA), adenosine monophosphate (AMP), adenosine, ammonium hydroxide (25–28%), zirconium chloride ($ZrCl_4$), 2-amino-1,4-benzenedicarboxylic acid, *N,N*-dimethylformamide (DMF) and HCl were purchased from J&K scientific (Shanghai, China). Methanol, ethanol, 2,2-azobisisobutyronitrile (AIBN, recrystallized in methanol before use) were purchased from Nanjing Chemical Reagent (Nanjing, China). Fused-silica capillaries with 150 μm I.D. and 375 μm O.D. were purchased from Yongnian Optic Fiber Plant (Hebei, China). Ultrapure water, purified with a Milli-Q Advantage A10 (Millipore, Milford, MA, USA), was used to prepare all solutions. Other chemical reagents were of analytical grade.

The chromatographic separations were performed on a TriSep 2100 system (Unimicro Technologies, Pleasanton, CA, USA). Scanning-electron microscopic (SEM) characterization was performed on a Hitachi FE-SEM S-4800 (Tokyo, Japan). Transmission electron microscopy (TEM) characterization was performed on a JEM-2100 system (JEOL, Tokyo, Japan). Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromerities, Norcross, GA, USA). The surface area and the micropore volume were calculated by data the Brunauer-Emmett-Teller (BET) method using adsorption. The X-ray diffraction (XRD) patterns of samples were acquired on an ARL XTRA diffractometer (Thermo Fisher Scientific, Waltham, MA) with Cu Kα radiation in the 2θ range of 5–50°. MALDI-TOF MS analyses were carried out on a 4800 plus MALDI TOF/TOF Analyzer (Applied Biosystems, Framingham, MA, USA) with a pulsed nitrogen laser operated at 337 nm. The laser energy was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratio (S/N). All mass spectra reported were obtained in the positive ion mode. The instrument was operated in reflectron mode for peptide detection. A typical spectrum was obtained by averaging 3000